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(54) Title: MICROFLUIDIC SURFACES

(57) Abstract: A microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures manufactured in the surface of a planar substrate. The device is characterized in that a part surface of at least one of the microchannel structures has a coat exposing a non-ionic hydrophilic polymer. The non-ionic hydrophilic polymer is preferably attached covalently directly to the part surface or to a polymer skeleton that is attached to the surface.

MICROFLUIDIC SURFACES

Technical field

The invention concerns a microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures fabricated in the surface of a planar substrate.

By the term "covered" is meant that a lid covers the microchannel structures thereby minimising or preventing undesired evaporation of liquids. The cover/lid may have microstructures matching each microchannel structure in the substrate surface.

The term "fabricated" means that two-dimensional and/or three-dimensional microstructures are present in the surface. The difference between a two-dimensional and a three-dimensional microstructure is that in the former variant there are no physical barriers delineating the structure while in the latter variant there are. See for instance WO 9958245 (Larsson et al).

The part of the cover/lid, which is facing the interior of a microchannel is included in the surface of a microchannel structure.

The planar substrate typically is made of inorganic and/or organic material, preferably of plastics. For examples of various inorganic and organic materials see under the heading "Material in the microfluidic device".

A microfluidic device encompasses that there is a liquid flow that causes mass transport of solutes and/or particles dispersed in the liquid from one functional part of the

structure to another. Sole capillaries, possibly with an area for application and an area for detection, as used in capillary electrophoresis in which solutes are caused to migrate by an applied electric field for separation purposes are not microfluidic devices as contemplated in the context of the invention. An electrophoresis capillary may, however, be part of a microfluidic device if the capillary is part of a microchannel structure in which there are one or more additional functional parts from and/or to which mass transport of a solute by a liquid flow is taking place as defined above.

The liquid is typically polar, for instance aqueous such as water.

Technical background.

Microfluidic devices require that liquid flow easily pass through the channels and that non-specific adsorption of reagents and analytes should be as low as possible, i.e. insignificant for the reactions to be carried out.

Reagents and/or analytes includes proteins, nucleic acids, carbohydrates, cells, cell particles, bacteria, viruses etc. Proteins include any compound exhibiting poly- or oligopeptide structure.

The hydrophilicity of surfaces within microchannel structures shall support reproducible and predetermined penetration of an aqueous liquid into the various parts of a structure. It is desirable that once the liquid has passed a possible break at the entrance of a part of the structure then the liquid spontaneously shall enter the part by capillary action (passive movement). This in turn means that the hydrophilicity of the surfaces within microchannel structures becomes of

increasing importance when going from a macroformat to a microformat.

From our experience, water contact angles around 20 degrees or lower may often be needed to accomplish reliable passive fluid movement into microchannel structures. However, it is not simple to manufacture surfaces which permanently have such low water contact angles. There is often a tendency for a change in water contact angles during storage, which renders it difficult to market microfluidic devices having standardised flow properties.

The situation is complicated by the fact that methods for preparing surfaces with very low water contact angles do not necessarily reduce the ability to non-specifically adsorb reagents and sample constituents. The surface/volume ratio increases when going from a macroformat down to smaller formats. This means that the capacity for non-specific adsorption of a surface increases inversely with the volume surrounded by the surface. Non-specific adsorption therefore becomes more critical in microformat devices than in larger devices.

An unacceptable non-specific adsorption of biomolecules is often associated with the presence of hydrophobic surface structures. This particular problem therefore is often more severe in relation to surfaces made of plastics and other hydrophobic materials compared to surfaces of native silicon surfaces and other similar inorganic materials.

There are a number of methods available for treating surfaces to make them hydrophilic in order to reduce non-specific adsorption of various kinds of biomolecules and other reagents. However, these methods generally do not concern balancing a low non-specific adsorption with a reliable and

reproducible liquid flow when miniaturizing macroformats down into microformats. Compare for instance Elbert et al., (Annu. Rev. Mater. Sci. 26 (1996) 365-394).

Surfaces that have been rendered repelling for biopolymers in general by coating with adducts between polyethylenimines and hydrophilic polymers have been described during the last decade (Brink et al (US 5,240,994), Bergström et al., US 5,250,613; Holmberg et al., J. Adhesion Sci. Technol. 7(6) (1993) 503-517; Bergström et al., Polymer Biomaterials, Eds Cooper, Bamfors, Tsuruta, VSP (1995) 195-204; Holmberg et al., Mittal Festschrift, Eds Van Ooij, Anderson, VSP 1998, p 443-460; and Holmberg et al., Biopolymers at Interfaces, Dekker 1998 (Surfactant Science Series 75), 597-626). Sequential attachment of a polyethylenimine and a hydrophilic polymer has also been described (Kiss et al., Prog. Colloid Polym. Sci. 74 (1987) 113-119).

Non-specific adsorption and/or electroendosmosis have been controlled in capillary electrophoresis by coating the inner surface of the capillary used with a hydrophilic layer, typically in form of a hydrophilic polymer (e.g. van Alstine et al US 4,690,749; Ekström & Arvidsson WO 9800709; Hjertén, US 4,680,201 (poly methacrylamide); Karger et al., US 5,840,388 (polyvinyl alcohol (PVA)); and Soane et al., US 5,858,188 and US 6,054,034 (acrylic microchannels). Capillary electrophoresis is a common name for separation techniques carried out in a narrow capillary utilizing an applied electric filed for mass transport and separation of the analytes.

Larsson et al (WO 9958245, Amersham Pharmacia Biotech) presents among others a microfluidic device in which microchannels between two planar substrates are defined by the interface between hydrophilic and hydrophobic areas in at

least one of the substrates. For aqueous liquids the hydrophilic areas define the fluid pathways. Various ways of obtaining a pattern of hydrophobic and hydrophilic surfaces for different purposes are discussed, for instance, plasma treatment, coating a hydrophobic surfaces with a hydrophilic polymer etc. The hydrophilic coat polymers suggested may or may not have aryl groups suggesting that Larsson et al are not focusing on lowering the water contact angle as much as possible or avoiding non-specific adsorption.

Larsson, Ocklind and Derand (PCT/EP00/05193 claiming priority from SE 9901100-9, filed 1999-03-24) describe the production of highly hydrophilic surfaces made of plastics. The surfaces retain their hydrophilicity even after being in contact with aqueous liquids. An additional issue in PCT/EP00/05193 is to balance a permanent hydrophilicity with good cell attachment properties. The surfaces are primarily suggested to be used in microfabricated devices.

Polyethylene glycol has been linked directly to the surface of a microchannel fabricated in silicone for testing the ability of polyethylyne glycol to prevent protein adsorption. See Bell, Brody and Yager (SPIE-Int. Soc. Opt. Eng. (1998) 3258 (Micro- and Nanofabricated Structures and Devices for Biomedical Environmental Applications) 134-140).

The objectives of the invention.

A first objective is to accomplish a sufficiently reliable and reproducible mass transport of reagents and sample constituents (e.g. analytes) in microfluidic devices.

A second objective is to enable a reliable and reproducible aqueous liquid flow in the microfluidic devices.

A third objective is to optimise non-specific adsorption and hydrophilicity in relation to each other for surfaces of fluid pathways in microfluidic devices.

The invention

We have discovered that by attaching a hydrophilic non-ionic polymer to the surface of a microchannel structure in a microfluidic device one can easily minimize the above-mentioned problems also for the most critical surface materials. This discovery facilitates creation of surfaces that permit reliable and reproducible transport of reagents and sample constituents in microfluidic devices.

The main aspect of the invention is a microfluidic device as defined under the heading "Technical Field". The characterizing feature is that at least a part surface of each microchannel structure exposes a firmly attached non-ionic hydrophilic polymer to the interior of the structure.

The non-ionic hydrophilic polymer may be attached directly to the surface of the microchannel structure or via a polymer skeleton that in turn is attached to the surface via multipoint attachment.

The non-ionic hydrophilic polymer

The non-ionic hydrophilic polymer contains a plurality of hydrophilic neutral groups. Neutral groups excludes non-charged groups that can be charged by a pH-change. Typical neutral hydrophilic groups contains an heteroatom (oxygen, sulphur or nitrogen) and may be selected among hydroxy, ether such as ethylene oxy (e.g. in polyethylene oxide), amides that may be N-substituted etc. The polymer as such is also inert towards the reagents and chemicals that are to be used in the microfluidic device.

Illustrative non-ionic hydrophilic polymers are preferably water-soluble when not bound to a surface. Their molecular weight is within the range from about 400 to about 1,000,000 daltons, preferably from about 1,000 to about 2000,000, such as below 100,000 daltons.

Non-ionic hydrophilic polymers are illustrated with polyethylene glycol, or more or less randomly distributed or block-distributed homo- and copolymers of lower alkylene oxides (C_{1-10}, such as C_{2-10}) or lower alkylene (C_{1-10}, such as C_{2-10}) bisepoxides in which the epoxide groups are linked together via a carbon chain comprising 2-10 sp^3-carbons. The carbon chain may be interrupted at one or more positions by an ether oxygen, i.e. an ether oxygen is inserted between two carbon atoms. A hydrogen atom at one or more of the methylene groups may be replaced with hydroxy groups or lower alkoxy groups (C_{1-4}). For stability reasons at most one oxygen atom should be bound to one and the same carbon atom.

Other suitable non-ionic hydrophilic polymers are polyhydroxy polymers that may be completely or partly natural or completely synthetic.

Completely or partly natural polyhydroxy polymers are represented by polysaccharides, such as dextran and its water-soluble derivatives, water-soluble derivatives of starch, and water-soluble derivatives of cellulose, such as certain cellulose ethers. Potentially interesting cellulose ethers are methyl cellulose, methyl hydroxy propyl cellulose, and ethyl hydroxy ethyl cellulose.

Synthetic polyhydroxy polymers of interest are also polyvinyl alcohol possibly in partly acetylated form, poly(hydroxy lower alkyl vinyl ether) polymers, polymers obtained by

polymerisation of epichlorohydrin, glycidol and similar bifunctionally reactive monomers giving polyhydroxy polymers.

Polyvinylpyrrolidone (PVP), polyacrylamides, polymethacrylamides etc are examples of polymers in which there are a plurality of amide groups.

Further suitable hydrophilic polymers are reaction products (adducts) between ethylene oxide, optionally in combination with higher alkylene oxides or bisepoxides, or tetrahydrofuran, and a dihydroxy or polyhydroxy compound as illustrated with glycerol, pentaerythritol and any of the polyhydroxy polymers referred to in the preceding paragraphs.

The non-ionic hydrophilic polymer may have the same structure as described for the extenders defined in Berg et al (WO 9833572) which is hereby incorporated by reference. In contrast to Berg et al there is no imperative need for the presence of an affinity ligand on the hydrophilic polymer used in the present invention.

One or more positions in the non-ionic hydrophilic polymer may be utilized for attachment. In order to make the hydrophilic polymer flexible the number of attachment points should be as low as possible, for instance one, two or three positions per polymer molecule. For straight chain polymers, such as lower alkylene oxide polymers similar to polyethylene oxide, the number of attachment points is typically one or two, with preference for one.

Depending on the position of a coated part surface within a microchannel structure, the hydrophilic polymer may carry an immobilized reactant (often called ligand when affinity reactions are concerned). Depending on the particular use of a microchannel structure such reactants can be so called

affinity reactants that are used to catch an analyte or an added reactant or a contaminant present in the sample. Immobilized ligands also include immobilized enzymes. According to the invention this kind of reactants are preferably present in reaction chambers/cavities (see below).

The skeleton

The skeleton may be an organic or inorganic cationic, anionic or neutral polymer of inorganic or organic material.

With respect to inorganic skeletons, the preferred variants are polymers such as silicon oxide. See the experimental part.

With respect to organic skeletons, the preferred variants are cationic polymers, such as a polyamine, i.e. a polymer containing two or more primary, secondary or tertiary amine groups or quaternary ammonium groups. The preferred polyamines are polyalkylenimines, i.e. polymers in which amine groups are interlinked by alkylene chains. The alkylene chains are for instance selected among C_{1-6} alkylene chains. The alkylene chains may carry neutral hydrophilic groups, for instance hydroxy (HO) or poly (including oligo) lower alkylene oxy groups [-O-$((C_2H_4)_nO)_mH$ where n is 1-5 and m is from 1 and upwards for instance ≤ 100 or ≤ 50)], amide groups, acyl, acyloxy, lower alkyl (for instance C_{1-5}) and other neutral groups and/or groups that are unreactive under the conditions to be applied in the microfluidic device.

The preferred molecular weight of the skeleton including polyamine skeletons is within the range of 10,000-3,000,000 daltons, preferably about 50,000-2,000,000 daltons. The structure of the skeleton can be linear, branched, hyperbranched or dendritic. The preferred polyamine skeleton is polyethylenimine, a compound that is achievable e.g. by

polymerizing ethylene imine, usually giving hyperbranched chains.

Attachment of the non-ionic hydrophilic polymer

The introduction of the non-ionic hydrophilic polymer groups on the channel surfaces may be done according to principles well-known in the field, forinstance by directly attaching the hydrophilic polymer to the desired part surface or via the kind of skeleton discussed above. The adduct between the skeleton and the non-ionic hydrophilic polymer may be (i) formed separately before it is attached to the surface or (ii) on the surface by first attaching the skeleton and then the hydrophilic polymer. Alternative (ii) can be carried out by (a) grafting a preprepared non-ionic hydrophilic polymer to the skeleton or (b) graft polymerisation of suitable monomers.

Both the non-ionic hydrophilic polymer and the skeleton may be stabilized to the underlying surfaces via covalent bonds, electrostatic interaction etc and/or by cross-linking *in situ* or afterwards. A polyamine skeleton, for instance, may be attached covalently by reacting its amine functions with aminereactive groups that are originally present or have been introduced on the uncoated substrate surface.

It is important that the nude part surface to be coated according to the invention has groups, which enable stable interaction between the non-ionic hydrophilic polymer and the surface and between the skeleton and the surface. Cationic skeletons, for instance polyamines, require that negatively charged or chargeable groups or groups otherwise capable of binding to amine groups, typically hydrophilic, are exposed on the surface. Polar and/or charged or chargeable groups may easily be introduced on plastics surfaces, for instance by treatment with O_2- and acrylic acid-containing plasmas, by oxidation with permanaganate or bichromate in concentrated sulphuric acid, by coating with polymers containing these type

of groups etc. In other words by techniques well-known in the scientific and patent literature. The plastics surface as such may also contain this kind of groups without any pretreatment, i.e. by being obtained from polymerisation of monomers either carrying the above-mentioned type of groups or groups that subsequent to polymerisation easily can be transformed to such groups.

If the surface to be coated is made of a metal, for instance of gold or platina, and the non-ionic hydrophilic polymer or skeleton has thiol groups, attachment can be accomplished via bonds that are partly covalent.

If the non-ionic hydrophilic polymer or the skeleton have hydrocarbon groups, for instance pure alkyl groups or phenyl groups, one can envisage that attachment to the substrate surface can take place via hydrophobic interactions.

Water contact angles

The optimal water contact angle depends on the analyses and reactions to be carried out in the microchannel structure, dimensions of the microchannels and chambers of the structures, composition and surface tension of liquids used, etc. As a rule of thumb, the inventive coat should be selected to provide a water contact angle that is $\leq 30°$, such as $\leq 25°$ or $\leq 20°$. These figures refer to values obtained at the temperature of use, primarily room temperature.

So far the most superior surfaces have been those based on adducts between polyethylene imine and polyethylene glycol with monosite (mono group terminal) attachment of the non-ionic hydrophilic polymer to the polyethylene imine skeleton. The best mode to date of this preferred variant is given in the experimental part (example 1).

Thickness of the coat

The thickness of the hydrated coat provided by the non-ionic hydrophilic polymers should be ≤ 50 %, for instance ≤ 20 % of the smallest distance between two opposing sides of a part of the microchannel structure comprising the surface coated according to the invention. This typically means that an optimal thickness will be within the interval 0.1-1000 nm, for instance 1-100 nm, with the provision that the coat shall permit a desired flow to pass through.

Structures in the microfluidic device.

The microfluidic device may be disc-formed of various geometries, with the round form being the preferred variant (CD-form).

On devices having round forms, the microchannel structures may be arranged radially with an intended flow direction from an inner application area radially towards the periphery of the disc. In this variant the most practical ways of driving the flow is by capillary action, centripetal force (spinning the disc) and/or hydrodynamically.

Each microchannel structure comprises one or more channels and/or one or more cavities in the microformat. Different parts of a structure may have different discrete functions. Thus there may be one or more parts that function as (a) application chamber/cavity/area (b) conduit for liquid transport, (c) reaction chamber/cavity, (d) volume defining unit, (e) mixing chamber/cavity, (f) chamber for separating components in the sample, for instance by capillary electrophoresis, chromatography and the like (g) detection chamber/cavity, (h) waste conduit/chamber/cavity etc. According to the invention at least one of these parts may

have the inventive coat on its surface, i.e. corresponds to the part surface discussed above.

When the structure is used, necessary reagents and/or sample including the analyte are applied to an application area and transported downstream in the structure by an applied liquid flow. Some of the reagents may have been predispensed to a chamber/cavity. The liquid flow may be driven by capillary forces, and/or centripetal force, pressure differences applied externally over a microchannel structure and also other non-electrokinetic forces that are externally applied and cause transport of the liquid and the analytes and reagents in the same direction. The liquid flow may also be driven by pressure generated by electroendoosmosis created within the structure. The liquid flow will thus transport reagents and analytes and other constituents from an application area/cavity/chamber into a sequence comprising a particular order of preselected parts (b)-(h). The liquid flow may be paused when a reagent and/or analyte have reached a preselected part in which they are subjected to a certain procedure, for instance capillary electrophoresis in a separation part, a reaction in a reaction part, detection in a detection part etc.

Analytical and preparative methods as discussed below utilizing the microfluidic device of the invention with transport of liquid, reagents and analytes as described in the preceding paragraph constitute a separate aspect of the invention.

Microformat means that at least one liquid conduit in the structure has a depth and/or width that is in the microformat range, i.e. $< 10^3$ µm, preferably $< 10^2$ µm. Each microchannel structure extends in a common plane of the planar substrate material. In addition there may be extensions in other

directions, primarily perpendicular to the common plane. Such other extensions may function as sample or liquid application areas or connections to other microchannel structures that are not located in the common plane, for instance.

The distance between two opposite walls in a channel is ≤ 1000 μm, such as ≤ 100 μm, or even ≤ 10 μm, such as ≤ 1 μm. The structures may also contain one or more chambers or cavities connected to the channels and having volumes being ≤ 500 μl, such as ≤ 100 μl and even ≤ 10 μl such as ≤ 1 μl. The depths of the chambers/cavities may typically be in the interval ≤ 1000 μm such as ≤ 100 μm such as ≤ 10 μm or even ≤ 1 μm. The lower limit is always significantly greater than the largest of the reagents used. The lower limits of chambers and channels are typically in the range 0.1-0.01 μm for devices that are to be delivered in dry form.

It is believed that the preferred variants of the inventive microfluidic devices will be delivered to the customer in a dried state. The surfaces of the microchannel structures of the device therefore should have a hydrophilicity sufficient to permit the aqueous liquid to be used to penetrate the different parts of the channels of the structure by capillary forces (self-suction).

There may be conduits enabling liquid communication between individual microchannel structures within a set.

Material in the microfluidic device.

The surface to be coated according to the invention typically is made of inorganic and/or organic material, preferably of plastics. Diamond material and other forms of elemental carbon are included in the term organic material. Among suitable

inorganic surface materials can be mentioned metal surfaces, e.g. made of gold, platina etc.

Plastics to be coated according to the invention may have been obtained by polymerisation of monomers comprising unsaturation such as carbon-carbon double bonds and/or carbon-carbon-triple bonds.

The monomers may, for instance, be selected from mono-, di and poly/oligo-unsaturated compounds, e.g. vinyl compounds and other compounds containing unsaturation. Illustrative monomers are:

(i) alkenes/alkadienes (such as ethylene, butadiene, propylene and including substituted forms such as vinyl ethers), cycloalkenes, polyfluorovinyl hydrocarbons (for instance tetrafluoroethylene), alkene-containing acids, esters, amides, nitriles etc for instance various methacryl/acryl compounds; and

(ii) vinyl aryl compounds (such as mono-, di- and trivinyl benzenes) that optionally may be substituted with for instance lower alkyl groups (C1-6) etc.

Another type of plastics are based on condensation polymers in which the monomers are selected from compounds exhibiting two or more groups selected among amino, hydroxy, carboxy etc groups. Particularly emphasised monomers are polyamino monomers, polycarboxy monomers (including corresponding reactive halides, esters and anhydrides), poly hydroxy monomers, amino-carboxy monomers, amino-hydroxy monomers and hydroxy-carboxy monomers, in which poly stands for two, three or more functional groups. Polyfunctional compounds include compounds having a functional group that is reactive twice, for instance carbonic acid or formaldehyde. The plastics contemplated are typically polycarbonates, polyamides,

polyamines, polyethers etc. Polyethers include the corresponding silicon analogues, such as silicone rubber.

The polymers of the plastics may be in cross-linked form.

The plastics may be a mixture of two or more different polymer(s)/copolymer(s).

Particularly interesting plastics are those that have a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm. By non-significant fluorescence is meant that the fluorescence intensity in the above-given emission wavelength interval should be below 50 % of the fluorescence intensity for a reference plastics (= a polycarbonate of bisphenol A without fluorescent additives). In fact it does not harm in case the fluorescence intensity of the plastics is even lower, such as < 30 % or < 15 %, such as < 5 % or < 1 %, of the fluorescence intensity of the reference plastics. Typical plastics having an acceptable fluorescence are based on polymers of aliphatic monomers containing polymerizable carbon-carbon double bonds, such as polymers of cykloalkenes (e.g. norbornene och substituted norbornenes), ethylene, propylenes etc, as well as other non-aromatic polymers of high purity, e.g. certain grades of polymethylmethacrylate.

In preferred variants of the invention the same limits for fluorescence also apply to the microfluidic structure after having been coated in accordance with the invention.

Applications in which the inventive microfluidic device can be used.

The primary use of the microfluidic devices of the invention is in analytical and preparative chemical and biochemical systems.

Typical analytical systems in which the microfluidic systems described herein may comprise as the main steps one or more of (a) sample preparation, (b) assay reactions and (c) detection. Sample preparation means the preparation of a sample in order to make it suitable for the assay reactions and/or for the detection of a certain activity or molecular entity. This may for example mean that substances interfering with the assay reactions and/or detection is removed or otherwise neutralized, that substances are amplified and/or derivatized etc. Typical examples are (1) amplifying one or more nucleic acid sequences in a sample, for instance by polymerase chain reaction (PCR), (2) removing of species cross-reacting with an analyte in assays involving affinity reactions etc. Typical assay reactions are (i) reactions involving cells, (ii) affinity reactions, for instance biospecific affinity including immune reactions, enzymatic reactions, hybridization/annealing etc, (iii) precipitation reactions, (iv) pure chemical reactions involving formation or breaking up of covalent bonds, etc. The detection reaction may involve fluorometry, chemiluminometry, mass spectrometry, nephelometry, turbidometry etc. The detection reaction aims at detection of the result of the assay reaction(s) and at relating a found result with the qualitative or quantitative presence of an activity in the original sample. The activity can be a biological, a chemical, a biochemical etc activity. It may be as the presence of a compound as such or simply as an activity of a known or unknown compound. If the system is used for diagnostic purposes the result in the detection step is further correlated to the medicinal status of the individual from which the sample derives. The applicable analytical systems may thus comprise affinity assays, such as immune assays, hybridisation assays, cell biology assays, mutation detection, genome characterisation, enzyme assays, screening assays for finding new affinity pairs etc. Methods

for the analysis of sample content of proteins, nucleic acids, carbohydrates, lipids and other molecules with particular emphasis of other bio-organic molecules are also included.

The microfluidic device of the present invention may also find use for the set up of libraries of compounds including synthetic peptide and oligonucleotide libraries, for instance by solid phase synthesis. The synthesis of so called combinatorial libraries of compounds is also included.

The invention will now be described with reference to non-limitative experiments that function as proof of principle.

EXPERIMENTAL PART

A. Coat of PEG-PEI adduct

a. Synthesis of PEG-PEI adduct

0.43 g of polyethylenimine (Polymin SN from BASF, Germany) was dissolved in 45 ml of 50 mM sodium borate buffer (pH 9.5) at 45°C. 5 g of the glycidyl ether of monomethoxy polyethylene glycol (Mw 5 000) was added during stirring and the mixture was stirred for 3 h at 45°C.

b. Surface treatment

A polycarbonate CD disc (polycarbonate of Bisphenol A, Macrolon DP-1265, Bayer AG, Germany) with a recessed microchannel pattern was placed in a plasma reactor (Plasma Science PS0500, BOC Coating Technology, USA) and treated with an oxygen plasma at 5 sccm gas flow and 500 W RF power for 10 min. After venting the reactor, the disc was immersed in a 0.1% solution of the PEG-PEI adduct in borate buffer pH 9.5 for 1 h. The disc was then rinsed with distilled water, blown dry with nitrogen and the water contact angle (sessile drop) was measured on a Ramé-Hart manual goniometer bench. The average of six equilibrium measurements (three droplets) was

24 degrees. An XPS spectrum of the treated surface gave the following molar elemental composition: 73.2% C, 3.7 % N, 23.1% O, showing that the surface was essentially covered by the adsorbed PEG-PEI adduct.

c. Capillary wetting

Another polycarbonate CD disc of the same material as above with a recessed microchannel pattern was treated as in example 2. It was then covered with a thin silicone rubber lid, with a hole placed over a microchannel. When a droplet of water was placed in the hole with a micropipette, the water was drawn in by capillary forces and penetrated the entire accessible channel system.

d. Comparative examples of surface treatments

a) A polycarbonate disc of the same material as above with a recessed microchannel pattern was dipped into a 0.5% water solution of phenyl dextran (degree of substitution: 0.2 per monosacharide unit of dextran, Mw 40 000) for 1 h. After water rinsing, the disc was blown dry with nitrogen. The water contact angle was 30 degrees. When a silicone rubber lid was placed over the disc with a hole over a channel, the droplet was not spontaneously drawn in. When a vacuum was applied to the channel through another hole in the lid, the droplet could however be introduced by suction.

b) A polycarbonate disc of the same material as above with a recessed microchannel pattern was immersed over night in a 1 % water solution of a polyethylene glycol "polypropylene glycol" polyethylene glycol triblock copolymer (Pluronic F108 from BASF). After water rinsing the disc was blown dry with nitrogen. The water contact angle was 60 degrees. When a silicone rubber lid was placed over the disc with a hole over a channel, the droplet was not spontaneously drawn in. When a vacuum was applied to the channel through another

hole in the lid, the droplet could however be introduced by suction.

B. POLY(ACRYLAMIDE) COATING.

a) Activation of the surface.

A PET foil (polyethylene terephthalate, Melinex®, ICI), evaporation coated with a thin film of silicon oxide, was used as a lid. The silicon oxide side of the PET foil was washed with ethanol and thereafter UV/Ozone (UVO cleaner, Model no 144A X-220, Jelight Company, USA) treated for 5 minutes. 15 mm Bind silane (3-methacryloloxypropyl trimethoxysilane, Amersham Pharmacia Biotech), 1.25 ml 10% acetic acid and 5 ml ethanol was mixed and thereafter applied onto the foil using a brush. After evaporation of the solvent, the foil was washed with ethanol and blown dry with nitrogen. The water contact angle (sessile drop) was measured on a Ramé-Hart manual goniometer. The average of repeated measurements was 62 degrees.

b. Grafting polyacrylamide to the activated surface

8.5 ml of 3 M acrylamide in water and 1.5 ml of 100 mM Irgacure 184 (dissolved in ethylene glycol, Ciba-Geigy) was mixed. The resulting solution was spread out on a quartz plate, and the activated PET foil was placed on top. The monomer solution was UV illuminated for 20 minutes through the quartz plate. The PET foil was then washed thoroughly in water and the average contact angle of repeated measurements was 17 degrees.

c. Capillary wetting

A piece of room temperature vulcanizing silicone rubber (Memosil, Wacker Chemie) having a microchannel structure and two holes was placed onto the polyacrylamide grafted PET foil (lid) (according to b above). When a droplet of water was

placed in the hole with a micropipette, the water was drawn in by capillary forces.

d. Comparative example of capillary wetting

A piece of room temperature vulcanizing silicone rubber (Memosil, Wacker Chemie) having a microchannel pattern and two holes were placed onto the activated PET foil (lid) (according to a above). When a droplet of water was placed in the hole with a micropipette, no water was drawn in by capillary forces. When vacuum was applied to the channel through the other hole, the droplet was sucked into the channel.

C L A I M S

1. A microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures manufactured in the surface of a planar substrate, **characterized** in that a part surface of at least one of the microchannel structures has a coat exposing a non-ionic hydrophilic polymer that preferably is attached covalently directly to the surface or to a polymer skeleton that is attached to the surface.

2. The microfluidic device of claim 1, **characterized** in that the surface of the planar substrate is made of plastics.

3. The microfluidic device according to any of claims 1-2, **characterized** in that the non-ionic hydrophilic polymer is attached to the polymer skeleton that is attached to the part surface, said skeleton preferably being branched and/or preferably being a polyamine.

4. The microfluidic device according to any of claims 1-3, **characterized** in that the substrate surface without the coat is made of plastics and that said part surface without coat is hydrophilized by plasma treatment or by an oxidation agent in order to introduce functional groups that allow for a subsequent attachment of the coat onto said part surface.

5. The microfluidic device according to any of claims 1-4, **characterized** in that the non-ionic hydrophilic polymer comprises one or more blocks of polyoxyethylene chains, with preference for the polymer being polyethylene glycol covalently attached at one of its ends to the skeleton or directly to the part surface and possibly having the remaining hydroxy group etherified.

6. The microfluidic device according to any of claims 1-6, **characterized** in that the hydrophilic non-ionic polymer is a polyethylene glycol, preferably a monoalkoxy variant such as the monomethoxy variant, which is attached to said part surface via the polymer skeleton which preferably is a polyethylenimine.

7. The microfluidic device according to any of claims 1-6, **characterized** in that the hydrophilic non-ionic polymer is attached to said part surface or to said polymer skeleton via one-point attachment, preferably covalently.

8. The microfluidic device according to any of claims 2-7, **characterized** in that the plastics has a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm.

9. The microfluidic device according to any of claims 1-3 and 5-8, **characterized** in that said polymer skeleton is an inorganic or an organic polymer.

10. The microfluidic device according to any of claims 1-4 and 7-9, **characterized** in that said non-ionic hydrophilic polymer comprises a plurality of amide bonds, e.g. is polymerisate/copolymerisate with monomers at least selected from acrylamide, methacrylamide, vinylpyrrolidone etc.

11. The microfluidic device according to any of claims 1-10, **characterized** in that it is in a dried state that is capable of being rehydrated.

12. The use of the microfluidic device according to any of claims 1-11 in analytical systems in which an assay comprising one or more of the steps:

(a) sample preparation,

(b) assay reaction and

(c) detection,

at least one and preferably more than two of said steps being carried out within the microfluidic device.

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/EP 00/12478

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B. FIELDS SEARCHED

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IPC 7 B01L B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	DE 197 53 847 A (ROCHE DIAGNOSTICS GMBH) 10 June 1999 (1999-06-10) abstract; figure 1 column 3, line 67 -column 4, line 60 column 9, line 50 -column 10, line 33	1-3,7, 11,12
Y		4
A	---	5,6,8-10
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Int. ional Application No
PCT/EP 00/12478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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PCT/EP 00/12478

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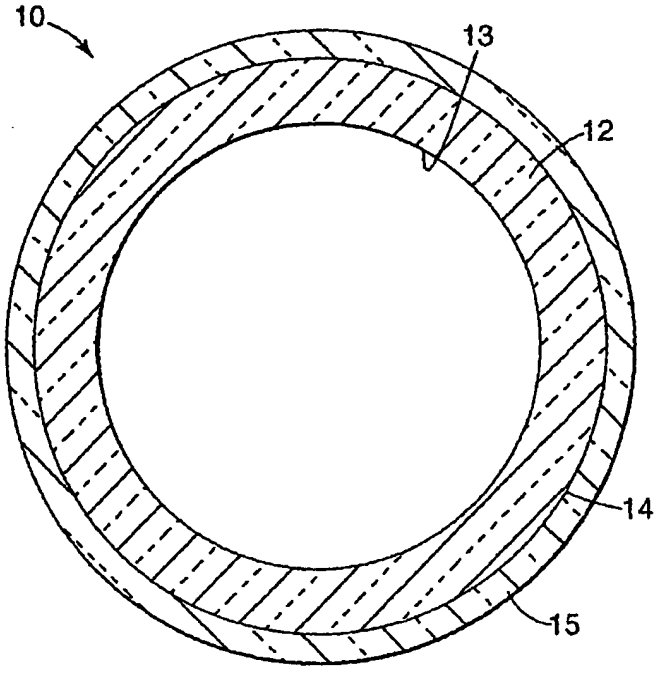
(54) Title: FLUID HANDLING DEVICES WITH DIAMOND-LIKE FILMS



(57) Abstract: Fluid handling devices including a substrate with a diamond-like film. The devices include capillaries and microfluidic articles.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

FLUID HANDLING DEVICES WITH DIAMOND-LIKE FILMS

This invention relates to fluid handling devices, such as microfluidic articles, including surfaces with diamond-like films thereon.

Silica capillaries are used extensively in electrophoresis, gas chromatography, electrochromatography, microbore liquid chromatography, and other chemical analytical techniques. Optical detection methods such as UV absorbance and fluorescence are often used in electrophoresis, electrochromatography, and liquid chromatography. The optical properties of silica are generally ideal for these detection methods; however, the use of pure uncoated silica capillaries is not possible because the lack of a protective coating causes the capillaries to be extremely fragile. As such, uncoated silica capillaries frequently will break under normal handling conditions.

Because of this, a protective coating must be put on the capillaries during fabrication. Conventionally, a polyimide coating is used. This coating has excellent thermal properties and gives the capillary excellent strength so that it can be easily handled; however, it is opaque and highly fluorescent and thus it is necessary to remove this coating from the portion of the capillary that is in an optical detector. Removal is somewhat difficult and it renders that portion of the capillary very delicate and easily broken.

There has also been a drive towards reducing the size of instrumentation used for analyzing and otherwise manipulating fluid samples such as biological fluid samples. The reduced size offers several advantages, including the ability to analyze very small samples, increased analytical speed, the ability to use reduced amounts of reagents, and reduced overall cost.

Various devices for microfluidic applications have been proposed. These devices typically include a glass or silicon substrate having a lithographically patterned and etched surface provided with one or more structures forming a microfluidic handling architecture. Plastic substrates such as polyimides, polyesters, and polycarbonates have been proposed as well; however, such plastic materials typically do not wet well and lack an electroosmotic flow necessary for the flow of liquid through the microchannels of the microfluidic handling architecture.

The present invention provides capillaries and other fluid handling devices, such as microfluidic articles, that include diamond-like films, preferably optically transmissive and/or hydrophilic diamond-like films. The articles of the present invention provide several advantages. For example, in the case of capillaries, optically transmissive diamond-like films do not necessarily have to be removed for detection. Hydrophilic diamond-like films provide good wetting and flow characteristics. For certain embodiments, particularly for certain microfluidic articles, the use of attachment chemistries that are used in conventional glass systems provide advantage.

The present invention provides a fluid handling device that includes a substrate and a diamond-like film (preferably one that is optically transparent and/or hydrophilic) disposed on at least a portion of the substrate. "Disposed" as used herein, means that the film is directly in contact with the substrate, bound or otherwise, or the film is in contact with one or more intervening layers, bound or otherwise. Herein, a film, rather than a coating, is disposed on a substrate. "Coating" as used herein, generally refers to a material that is first applied to a solid substrate in a liquid state, then solidified by UV radiation (photopolymerizable), heat (thermoset), or by removing solvent molecules from the coating solution.

Preferably, the fluid handling device is a capillary having an internal surface (which is typically a fluid handling surface) and an external surface (which is typically a nonfluid handling surface), wherein at least a portion of at least one of the internal or external surfaces has an optically transmissive diamond-like film disposed thereon. Preferably, the external surface of the capillary has an optically transmissive diamond-like film disposed on at least a portion thereof.

In another preferred embodiment, the fluid handling device can be a microfluidic article having microfluidic handling architecture including a fluid handling surface with an optically transmissive and/or hydrophilic diamond-like film disposed on at least a portion thereof. "Microfluidic handling architecture" includes, without limitation, open and closed or covered microchannels, reservoirs, sample handling regions and combinations thereof. The architecture may also, or alternatively, include a non-fluid handling surface having an optically transmissive and/or hydrophilic diamond-like film disposed on at least a portion thereof. Preferably, at least a portion of the fluid handling surface includes a hydrophilic diamond-like film disposed thereon.

In a preferred embodiment, a microfluidic article includes a first polymeric substrate having a first major surface that includes a plurality of microfluidic handling architectures and a second major surface, wherein the article is in the form of a roll.

In another embodiment, the present invention provides a fluid handling device that includes a substrate and an optically transmissive and/or hydrophilic film including at least about 25 atomic percent carbon, from 0 to about 50 atomic percent silicon, and from 0 to about 50 atomic percent oxygen, on a hydrogen-free basis, disposed on at least a portion of the substrate. "Hydrogen-free basis" refers to the atomic composition of a material as established by a method such as Electron Spectroscopy for Chemical Analysis (ESCA), which does not detect hydrogen even if large amounts are present in the thin films.

In yet another embodiment, the present invention provides a fluid handling device that includes a substrate and a film including at least about 30 atomic percent carbon, at least about 25 atomic percent silicon, and less than about 45 atomic percent oxygen, on a hydrogen-free basis, disposed on at least a portion of the substrate. Preferably, the film is optically transparent, and more preferably hydrophilic.

In still another embodiment, a fluid handling device is provided that includes a microfluidic article that includes a microfluidic handling architecture including a non-fluid handling surface wherein at least a portion thereof has disposed thereon a diamond-like film that is optically transmissive, hydrophilic, or both.

The present invention provides a method of manufacturing a hydrophilic diamond-like film. The method includes treating a diamond-like film in an oxygen-containing plasma.

Various other features and advantages of the present invention should become readily apparent with reference to the following detailed description, examples, claims and appended drawings.

The present invention provides capillaries and microfluidic articles, as well as other fluid handling devices, and methods of manufacturing the same. For purposes of this invention, the following definitions shall have the meanings set forth.

"A" or "an" refers to one or more of the recited elements.

"Affix" shall include any mode of attaching reactants to a diamond-like film. Such modes shall include, without limitation, covalent and ionic bonding, adherence, such

as with an adhesive, physical entrapment, and adsorption. This may or may not require the use of linking agents.

"Analyte" shall mean a molecule, compound, composition or complex, either naturally occurring or synthesized, to be detected or measured in or separated from a sample of interest. Analytes include, without limitation, proteins, peptides, fatty acids, nucleic acids, carbohydrates, hormones, steroids, lipids, vitamins, bacteria, viruses, pharmaceuticals, and metabolites.

"Diamond-like film" refers to substantially or completely amorphous films including carbon, and optionally including one or more additional components selected from the group of hydrogen, nitrogen, oxygen, fluorine, silicon, sulfur, titanium, and copper. Other elements may be present in certain embodiments. The films may be covalently bonded in a random system or in an interpenetrating system, such as in an interpenetrating diamond-like nanocomposite (called DYLYN), as described, e.g., U.S. Pat No. 5,466,431. The amorphous diamond-like films of this invention may contain clustering of atoms that give it a short-range order but are essentially void of medium and long range ordering that lead to micro or macro crystallinity which can adversely scatter actinic radiation having wavelengths of from 180 nm to 800 nm. The term "amorphous" means a substantially randomly-ordered non-crystalline material having no x-ray diffraction peaks or modest x-ray diffraction peaks. When atomic clustering is present, it typically occurs over dimensions that are small compared to the wavelength of radiation.

"Hydrophilic" as it relates to a diamond-like film shall mean a diamond-like film having a water contact angle of about 50 degrees or less, and preferably about 30 degrees or less.

"Linking agent" shall mean any chemical species capable of affixing a "Reactant" to the diamond-like film. Linking agents can be covalently bonded to the diamond-like film or provided by a polymeric coating thereon.

"Optically transmissive" as it relates to a film refers to the film having an extinction coefficient of no greater than 0.3 at 500 nanometers (nm). Preferably, the extinction coefficient is no greater than 0.010 at 250 nm.

"Reactant" shall mean any chemical molecule, compound, composition or complex, either naturally occurring or synthesized, that is capable of binding an analyte in a sample of interest either alone or in conjunction with a molecule or compound that

assists in binding the analyte to the diamond-like film, such as, for example, a coenzyme. The reactants of the present invention are useful for chemical or biochemical measurement, detection or separation. Accordingly, the term "Reactant" specifically excludes molecules, compounds, compositions or complexes, such as ink, that do not bind analytes as described above. Examples of reactants include, without limitation, polypeptides (e.g., proteins such as enzymes and antibodies), polynucleotides (e.g., oligonucleotides and cDNA), and carbohydrates.

Figure 1 is a cross-section of a capillary showing a diamond-like film on the external surface of the capillary.

Figure 2 is a perspective view of a microfluidic article showing a diamond-like film on a non-fluid handling surface of the article.

Figure 3 is a perspective view of an alternative microfluidic article showing a diamond-like film on a fluid handling surface of the article.

Figure 4 is a schematic plan view of a plasma reactor used to prepare samples as further described in the Examples.

Figure 5 is a schematic plan view of a plasma reactor used to prepare samples as further described in the Examples.

Figure 6 is a Weibull plot of glass capillaries including a diamond-like glass thin film further described in Example 5. A description of a Weibull plot may be found in 3M Technical Publication: Frederick Bacon, "Silica Optical Fibers – Application Note" available from 3M Optical Transport Systems, Connecticut.

Figure 7 depicts Raman spectra of the fluorescence measurements referenced in Figure 1.

The present invention provides capillaries and other fluid handling devices, such as microfluidic articles, having disposed on at least a portion thereof a diamond-like film, preferably one that is optically transmissive and/or hydrophilic. In the case of optically transmissive films, such films typically provide strength to the device, and preferably exhibit very low fluorescence. While providing strength, the films can also maintain a degree of flexibility. For fluid handling surfaces, hydrophilic diamond-like films can provide hydrophilic surfaces that enhance fluid transport. Furthermore, if desired, such films can include linking agents for affixing reactants or otherwise altering the surface

chemistry. The films can also function as a barrier to liquid evaporation and transmission through the substrate of which the device is made.

Referring to Figure 1, the present invention provides an exemplary capillary 10 that includes a substrate 12 with an internal surface 13 and an external surface 14, at least one of which has an optically transmissive diamond-like film 15 disposed thereon. The capillary can be made of glass or plastic. Typically, it is made of glass. According to the present invention, at least a portion of either the internal surface or the external surface, or both, has an optically transmissive diamond-like film thereon. Placing an optically transmissive diamond-like film on the the external surface 14 of a glass capillary eliminates the need for a polymeric coating, such as polyimide, to provide strength. Placing a diamond-like film of the external surface 14 of a plastic capillary reduces or prevents evaporation and transmission of the liquid, e.g., water, through the plastic substrate. Placing a diamond-like film on the internal surface 13 of a glass or plastic capillary provides the capability of varying the surface chemistry, and preferably provides a hydrophilic surface if a hydrophilic diamond-like film is used.

Referring to Figure 2, an exemplary microfluidic device is shown that is a single layer article 20 in the form of a sheet featuring a polymeric substrate (e.g., plastic substrate) 23 bearing a plurality of microfluidic handling architectures 24. The microfluidic handling architectures include a fluid handling surface 25. At least a portion of the fluid handling surface can include a diamond-like film that is either optically transmissive, hydrophilic, or both, disposed thereon, for similar reasons as described above for the capillaries. Significantly, for the fluid handling surfaces of such polymeric substrates, hydrophilic diamond-like films are preferred. Such hydrophilic diamond-like films, particularly, diamond-like glass films, can provide a surface that is more easily wettable and has a surface charge that allows electroosmotic flow that enhances fluid transport. With continuing reference to Figure 2, the article may optionally include a non-fluid handling surface 26, at least a portion of which may include a diamond-like film disposed thereon.

Referring to Figure 3, another exemplary embodiment of a microfluidic article 30 is shown that includes a first non-elastic (i.e., having insufficient elasticity in the direction normal to the plane of the substrate to act as a pump or valve when subjected to a cyclically varying force in that direction), polymeric substrate 28 having a first major

surface that includes a microfluidic handling architecture 24, and a second major surface, and a second polymeric substrate 32 that is integrally bonded (i.e., bonded directly to each other, as opposed to being bonded through an intermediate material such as an adhesive) to the second major surface of the first substrate. The second substrate is capable of forming a free-standing substrate in the absence of the first substrate. It provides mechanical support for the first substrate and also provides a means for incorporating additional features into the article such as microelectronic, microoptical, and/or micromechanical elements, thereby providing design flexibility. At least a portion of at least one of a surface, preferably a fluid handling surface, of the microfluidic handling architectures 24 has a hydrophilic diamond-like film disposed thereon. Preferably, the hydrophilic diamond-like film is also optically transmissive. The article preferably includes a cover layer overlying the microfluidic handling architecture. The cover layer, which may be bonded to the first surface of the first substrate, preferably is a polymeric layer.

In preferred embodiments, the diamond-like film can include linking agents and reactants thereon, as described more fully below. The linking agents are selected based on the reactants to be affixed to the film and the application for which the fluid handling device will be used.

Capillaries

A capillary is typically constructed of material that is sturdy and durable so that it can maintain its physical integrity through repeated use under normal conditions. It is typically constructed of nonconductive material. This is important for capillary electrophoresis, for example, so that high voltages can be applied across the capillary without generating excessive heat. Inorganic materials such as quartz, glass, fused silica, and organic materials such as polytetrafluoroethylene, fluorinated ethylene/propylene polymers, polyfluoroethylene, aramide, nylon (i.e., polyamide), polyvinyl chloride, polyvinyl fluoride, polystyrene, polyethylene, and the like, can be advantageously used to make capillaries.

The internal diameter (i.e., bore size) of the capillaries extends to a wide range of capillary sizes. In general, capillaries can range preferably from about 5 micrometers to

about 300 micrometers in internal diameter. The length of the capillary can range preferably from about 50 millimeters to about 30 meters.

The use of machined channels (e.g., capillary arrays) instead of individual capillary tubes are also known and are within the scope of fluid handling devices described herein. With conventional technology, however, multiple individual capillaries are still the more developed format. However, the films described herein can also be applied to such capillary arrays having machined channels.

Where excitation and/or detection are effected through the capillary wall, a particularly advantageous capillary is one that is constructed of transparent material. A transparent capillary that exhibits substantially no fluorescence, e.g., that exhibits fluorescence lower than background level, when exposed to the light used to irradiate a target species is especially useful in cases where excitation is effected through the capillary wall. Although such capillaries are known, the majority have a coating of an organic polymer (e.g., polyimide) that is opaque and highly fluorescent and thus must be removed from the portion of the capillary that is in an optical detector. Significantly, the optically transmissive diamond-like films of the present invention have substantially no fluorescence. Thus, these films need not necessarily be removed for optical detection of the samples contained in the capillaries.

Microfluidic Articles

Examples of microfluidic articles are described in Published International Patent Application Nos. WO 99/65542 and WO 99/65664, both published December 23, 1999, and U.S. Patent Nos. 5,637,469 to Wilding et al, and 5,842,787 to Kopf-Sill et al. Typically, microfluidic articles are polymer-based. Preferably, they can be produced efficiently in commercial-scale quantities, e.g., in the form of a roll good, and can be selectively tailored to perform a variety of functions, including analytical functions.

A preferred microfluidic article can be made by bringing a moldable material and the surface of an open molding tool (i.e., a molding tool that lacks a sealed cavity found in closed molds, of the type used in injection molding) into line contact (i.e., the point at which the tool contacts the moldable material as defined by a line that moves relative to both the tool and the moldable material) with each other to imprint, for example, a microfluidic processing architecture onto the moldable material, as described in Published

International Patent Application No. WO 99/65664, published December 23, 1999. The resulting molded article is then separated from the molding surface of the tool.

The moldable material can be an embossable polymeric substrate, a flowable resin composition, which can be cured upon exposure to thermal or actinic radiation prior to separating the molded article from the molding surface, or a molten thermoplastic composition which is cooled while in contact with the molding surface to solidify it.

Typically, a flowable resin composition is introduced onto a major surface of a polymeric substrate, and the substrate and molding tool are moved relative to each other to bring the tool and flowable resin composition into line contact with each other. The net result is a two-layer structure in which a microfluidic handling architecture-bearing layer is integrally bonded to the polymeric substrate.

Examples of suitable moldable materials include poly(methylmethacrylate) polycarbonates, polyesters, and polyimides. Examples of suitable photocurable resin compositions include alkyl acrylates and methacrylates (e.g., polymethyl methacrylate). Other ingredients which may be incorporated in the composition include photoinitiators, thixotropic agents, plasticizers, toughening agents, pigments, fillers, abrasive granules, stabilizers, light stabilizers, antioxidants, flow agents, bodying agents, flatting agents, colorants, binders, blowing agents, fungicides, bactericides, surfactants, glass and ceramic beads, and reinforcing materials such as woven and non-woven webs of organic and inorganic fibers.

A substrate may be bonded to the molded article to form a cover layer overlying the microfluidic handling architecture. Preferably, the substrate is a glass or polymeric substrate, although rigid cover layers such as glass cover layers may be used as well. Examples of suitable polymeric substrates include polycarbonate, polyester, poly(methylmethacrylate), polyethylene, and polypropylene. Bonding may be effected using an adhesive or by laminating or solvent welding the cover layer directly to the microfluidic handling architecture-bearing substrate. In addition, the cover layer may be part of the analytical instrumentation with which the article is designed to be used.

Significantly, diamond-like films described herein may be selectively patterned on portions of the microfluidic handling architectures, thereby forming discontinuous films. Deposition of diamond-like films may occur either in-line during manufacture or in a subsequent operation. The diamond-like films may perform a variety of functions. For

example, the films may be used to increase the hydrophilicity of the microfluidic handling architecture. They may reduce or prevent evaporation of the sample liquid. The diamond-like films, particularly the hydrophilic diamond-like glass films, may also facilitate wetting of the surfaces and enhance flow of the samples through the channels of the microfluidic handling architecture. They may also facilitate wicking a sizing gel into the microchannels of an electrophoresis device.

Layers of other inorganic materials may be selectively deposited on portions of the microfluidic handling architectures, as well, for example, using vacuum sputtering, electron beam deposition, solution deposition, or chemical vapor deposition. Such materials can be used to perform some of the same functions as that of the diamond-like films, and those that are conductive may also be used to form electrodes or diaphragms for piezoelectric or peristaltic pumping.

It is also possible to selectively deposit materials, such as reactants onto various portions of the microfluidic handling architecture. Alternatively, these materials may be deposited in a pre-determined pattern on the surface of the cover layer designed to contact the microfluidic handling architecture.

A microfluidic article can optionally include one or more microelectronic, microoptical, and/or micromechanical elements as well. Examples of microelectronic elements include conductive traces, electrodes, electrode pads, microheating elements, electrostatically driven pumps and valves, microelectromechanical systems (MEMS), and the like. Examples of microoptical elements include optical waveguides, waveguide detectors, reflective elements (e.g., prisms), beam splitters, lens elements, solid state light sources and detectors, and the like. Examples of micromechanical elements include filters, valves, pumps, pneumatic and hydraulic routing, and the like. The microelements may be incorporated in the cover layer, either surface of the microfluidic handling architecture-bearing substrate, an additional polymeric substrate bonded to the microfluidic handling architecture-bearing substrate, or a combination thereof.

Such articles can include a number of different microfluidic handling architecture designs. Accordingly, they can be used to perform numerous functions, including, for example, capillary array electrophoresis, kinetic inhibition assays, competition immunoassays, enzyme assays, nucleic acid hybridization assays, cell sorting, combinatorial chemistry, and electrochromatography.

The depth of a microchannel can be varied while maintaining a constant microchannel width. The microchannels can be used to construct vertically tapered inlet and outlet diffusers for a piezoelectric valve-less diffuser micropump, or used to provide electrokinetic zone control or electrokinetic focusing. Similarly, the width of a high aspect ratio microchannel can be tapered at constant depth. The resulting structure is also useful for providing electrokinetic zone control.

It is also possible to taper both the depth and width of the microchannels to provide a constant cross-sectional area or, alternatively, a constant cross-sectional perimeter. As a consequence of the constant cross-sectional area or perimeter, the resulting structure enables achievement of a constant voltage gradient throughout the length of the channel for predominantly electrophoretic flow or electroosmotic flow, thereby providing optical confinement for single molecule detection without loss of resolving power. This structure is also useful for providing a transition between low aspect ratio and high aspect ratio structures (e.g., high aspect ratio injection tees, low aspect ratio probe capture zones, microwell reactors, or piezoelectric drive elements) without loss of electrokinetic resolving power. It is also possible to prepare two intersecting microchannels having different depths. This feature, in turn, may be exploited to create a microfluidic switch in a hydrophobic substrate. Because of the depth difference, fluid in one arm of the relatively shallow microchannel will not cross the intersection unless a buffer is introduced into the relatively deeper microchannel to bridge the intersection. The variable depth feature is also useful for preparing post arrays for corralling probe capture beads in an immunoassay or nucleic acid assay, while simultaneously permitting the reporter reagent and fluid sample to flow freely.

Diamond-Like Films

Various diamond-like films are suitable for the present invention. Films typically include plasma and/or vapor deposited materials containing silicon atoms, such as silicon oxide films, silicon nitride films, silicon oxynitride films, plasma polymerized polysiloxane films, hydrogenated and nonhydrogenated amorphous silicon-containing films, silicon-doped diamond-like carbon films, and the like. See, for example, Applicants' Assignee's copending applications U.S. Serial No. 09/519449, filed on March

5, 2000, and U.S. Serial No. 09/519447, filed on March 5, 2000; and Plasma Deposited Thin Films, J. Fort & F. Jansen, Eds.; CRC Press, Boca Raton, FL (1986).

As the term is used herein, "diamond-like film" refers to substantially or completely amorphous films including carbon, and optionally including one or more additional components selected from the group of hydrogen, nitrogen, oxygen, fluorine, silicon, sulfur, titanium, and copper. Other elements may be present in certain embodiments.

As noted above and described below, the diamond-like films include approximately 25 to approximately 100 atomic percent carbon, with optional additional components making up the remainder (references to compositional percentages herein refer to atomic percents). The films may be covalently coupled or interpenetrating. The amorphous diamond-like films of this invention may contain clustering of atoms that give a short-range order but are essentially void of medium and long range ordering that lead to micro or macro crystallinity which can adversely scatter actinic radiation having wavelengths of from 180 nm to 800 nm.

Several special classes of covalently bonded diamond-like films are useful in this invention, as long as they are optically transmissive and/or hydrophilic. Diamond-like carbon (DLC) films, which include carbon and up to about 70% hydrogen, preferably about 10% to about 70%, typically are not optically transmissive, as defined herein.

Another class of suitable diamond-like films include diamond-like networks (DLN). In DLN, the amorphous carbon-based system is doped with other atoms in addition to hydrogen. These may include fluorine, nitrogen, oxygen, silicon, copper, iodine, boron, etc. DLN contains at least about 25% carbon. Typically the total concentration of these one or more additional elements is low (less than about 30%) in order to preserve the diamond-like nature of the films.

A particularly preferred class of diamond-like film materials is diamond-like glass (DLG), in which the amorphous carbon system includes a substantial quantity of silicon and oxygen, as in glass, yet still retains diamond-like properties. In these films, on a hydrogen-free basis, there is at least about 30% carbon, a substantial amount of silicon (at least about 25%) and not more than about 45% oxygen. The unique combination of a fairly high amount of silicon with a significant amount of oxygen and a substantial amount of carbon makes these films highly transparent and flexible (unlike glass). Furthermore,

DLG films can be surface modified in oxygen-containing plasma to produce hydrophilic surfaces that remain stable over time. This is a preferred film for use in the fluid handling devices of the present invention.

The diamond-like films typically include on a hydrogen-free basis at least about 25 atomic percent carbon, from 0 to about 50 atomic percent silicon, and from 0 to about 50 atomic percent oxygen. In certain implementations, the film includes from about 25 to about 70 atomic percent carbon, about 20 to about 40 atomic percent silicon, and about 20 to about 40 atomic percent oxygen. In another implementation, the film includes from about 30 to about 36 atomic percent carbon, from about 26 to about 32 atomic percent silicon, and from about 35 to about 41 atomic percent oxygen on a hydrogen-free basis.

In addition, a class of interpenetrating diamond-like films are useful in this invention. These diamond-like thin films are called DYLYN and are interpenetrating systems of two materials. These interpenetrating diamond-like thin films are disclosed in U.S. Pat. No. 5,466,431.

Diamond thin films having significantly different properties from the amorphous diamond-like film of the present invention due to the arrangement and intermolecular bonds of carbon atoms in the specific material, have previously been deposited on substrates. The type and amount of intermolecular bonds are determined by infrared (IR) and nuclear magnetic resonance (NMR) spectra. Carbon deposits contain substantially two types of carbon-carbon bonds: trigonal graphite bonds (sp^2) and tetrahedral diamond bonds (sp^3). Diamond is composed of virtually all tetrahedral bonds, while amorphous diamond-like films are composed of approximately 50% to approximately 90% tetrahedral bonds, and graphite is composed of virtually all trigonal bonds.

The crystallinity and the nature of the bonding of the carbonaceous film determines the physical and chemical properties of the deposit. Diamond is crystalline, whereas the amorphous diamond-like films of the invention are a non-crystalline, amorphous material, as determined by x-ray diffraction. Diamond is essentially pure carbon, whereas diamond-like films can contain a substantial amount of additional components (up to approximately 50 atomic percent for a single non-carbon component, and up to approximately 75 atomic percent for the combination of all additional non-carbon components). These atomic percents can be determined by combustion analysis.

Diamond has the highest packing density, or gram atom density (GAD), of any material at ambient pressure. Its GAD is 0.28 gram atoms/cc. Amorphous diamond-like films have a GAD ranging from about 0.20 to 0.28 gram atoms/cc. In contrast, graphite has a GAD of 0.18 gram atoms/cc. The high packing density of amorphous diamond-like films affords excellent resistance to diffusion of liquid or gaseous materials. Gram atom density is calculated from measurements of the weight and thickness of a material. "Gram atom" refers to the atomic weight of a material expressed in grams.

Amorphous diamond-like films are diamond-like because, in addition to the foregoing physical properties that are similar to diamond, they have many of the desirable performance properties of diamond such as extreme hardness (1000 to 2000 kg/mm^2), high electrical resistivity (10^9 to 10^{13} ohm-cm), a low coefficient of friction (0.1), and optical transparency over a wide range of wavelengths (an extinction coefficient of less than 0.1 in the 400 to 800 nanometer range).

Diamond films, as opposed to diamond-like films, also have some properties, which in many applications make them less beneficial as a protective layer than amorphous diamond-like films. Diamond films have grain structures, as determined by electron microscopy. The grain boundaries are a path for chemical attack and degradation of the substrates, and also cause scattering of actinic radiation. Amorphous diamond-like films do not have a grain structure, as determined by electron microscopy, and are thus well suited to applications wherein actinic radiation will pass through the film.

The polycrystalline structure of diamond films causes light scattering from the grain boundaries. Surprisingly, diamond-like films in accordance with the invention allow for excellent light transmission. Additionally, the visible light transmission of a carbon-, or carbon- and hydrogen-, based film is further improved by incorporating silicon and oxygen atoms into the amorphous diamond-like structure during the deposition process. This is not possible for crystalline diamond thin films because additional components will disrupt its crystalline lattice structure.

In creating a diamond-like film, various additional components can be incorporated into the basic amorphous carbon or carbon and hydrogen structure. These additional components can be used to alter and enhance the properties that the diamond-like film imparts to the substrate. For example, it may be desirable to further enhance the barrier and surface properties.

The additional components may include one or more of hydrogen (if not already incorporated), nitrogen, oxygen, fluorine, silicon, sulfur, titanium, or copper. Other additional components may also work well. The addition of hydrogen promotes the formation of tetrahedral bonds. The addition of fluorine is particularly useful in enhancing barrier and surface properties of the diamond-like film, including the ability to be dispersed in an incompatible matrix. The addition of silicon and oxygen tends to improve the optical transparency and thermal stability of the diamond-like film. The addition of nitrogen may be used to enhance resistance to oxidation and to increase electrical conductivity. The addition of sulfur can enhance adhesion. The addition of titanium tends to enhance adhesion as well as diffusion and barrier properties.

Diamond-like films can be deposited in a variety of thicknesses, depending on the deposition conditions and starting materials. For example, they can be a thin as about 10 Angstroms or as thick as about 10 micrometers (i.e., microns), if desired. Preferably, they are about 200 Angstroms thick to about 1 micron thick. More preferably, they are about 500 Angstroms thick to about 1000 Angstroms thick.

Adhesion of the diamond-like film to the substrate may be improved, if desired, by any of the methods known to one skilled in the art. These methods typically include various pre-treatments such as corona or plasma treatment.

In certain embodiments, diamond-like films, particularly hydrophilic diamond-like films, can include linking agents, and optionally reactants, to modify the chemistry of the surface of the fluid handling devices. The linking agents may be substantially over the entire area of a surface of the substrate, such as the major surface, or in spots that may be in a regular or irregular pattern on such surface. If desired, more than one type of linking agent may be on the substrate.

Reactants can be disposed on the diamond-like films, optionally through linking agents, to create binding sites. As described more fully below, with respect to the methods of the present invention, any number of processes known in the art may be used to introduce the reactants. It is understood that the mode of affixation may vary in accordance with the reactant or reactants employed.

The type of reactant used in the present invention will vary according to the application and the analyte of interest. For example, when characterizing DNA, oligonucleotides are preferred. When conducting diagnostic tests to determine the

presence of an antigen, antibodies are preferred. In other applications, enzymes may be preferred. Accordingly, suitable reactants include, without limitation, polypeptides (e.g., proteins such as enzymes and antibodies), polynucleotides (e.g., nucleic acids, oligonucleotides, cDNA), and carbohydrates. Preferred reactants include proteins, nucleic acids, and carbohydrates.

Method for Forming Diamond-Like Films

The diamond-like films are deposited by plasma deposition onto substrates from gases using the methods and apparatus disclosed in Applicants' Assignee's copending applications U.S. Serial No. 09/519449, filed on March 5, 2000, and U.S. Serial No. 09/519447, filed on March 5, 2000.

A typical system includes electrodes one or both of which are powered by RF and a grounded reaction chamber. A substrate is placed proximate the electrode and an ion sheath is formed around the powered electrode to establish a large electric field across the ion sheath. Plasma is generated and sustained by means of a power supply (an RF generator operating at a frequency in the range of about 0.001 Hz to about 100 MHz). To obtain efficient power coupling (i.e., wherein the reflected power is a small fraction of the incident power), the impedance of the plasma load can be matched to the power supply by means of matching network that includes two variable capacitors and an inductor, which is available from RF Power Products, Kresson, NJ, as Model # AMN 3000.

Briefly, the grounded reaction chamber is partially evacuated, and radio frequency power is applied to one of two electrodes. A carbon-containing source is introduced between the electrodes to form a plasma that includes reactive species in proximity to the electrodes, and to also form an ion sheath proximate at least one electrode. The substrate is exposed to the reactive species within the ion sheath that is proximate an electrode to form a diamond-like thin film on the substrate. The conditions can result in a thin film that includes a diamond-like covalent system that includes, on a hydrogen-free basis, at least 30 atomic percent carbon, from 0 to 50 atomic percent silicon, and from 0 to 50 atomic percent oxygen.

Deposition occurs at reduced pressures (relative to atmospheric pressure) and in a controlled environment. A carbon-rich plasma is created in a reaction chamber by applying an electric field to a carbon-containing gas. Substrates on which films are to be

deposited are usually held in a vessel or container in the reactor. Deposition of the diamond-like film typically occurs at rates ranging from about 1 nanometer per second (nm/second) to about 100 nm/second (about 10 Angstrom per second to about 1000 Angstroms per second), depending on conditions including pressure, power, concentration of gas, types of gases, relative size of electrodes, etc. In general, deposition rates increase with increasing power, pressure, and concentration of gas, but the rates will approach an upper limit.

Species within the plasma react on the substrate surface to form covalent bonds, resulting in an amorphous diamond-like film on the surface of the substrates. A multiplicity of substrates may simultaneously have a film deposited on them during the process of this invention. The substrates can be held in a vessel or container within an evacuable chamber that is capable of maintaining conditions that produce diamond-like film deposition. That is, the chamber provides an environment that allows for the control of, among other things, pressure, the flow of various inert and reactive gases, voltage supplied to the powered electrode, strength of the electric field across the ion sheath, formation of a plasma containing reactive species, intensity of ion bombardment and rate of deposition of a diamond-like film from the reactive species.

Prior to the deposition process, the chamber is evacuated to the extent necessary to remove air and any impurities. Inert gases (such as argon) may be admitted into the chamber to alter pressure. Once the substrate is placed in the chamber and it is evacuated, a substance containing carbon (and usually hydrogen), and optionally a substance from which an additional component can be deposited, is admitted into the chamber and, upon application of an electric field, forms a plasma from which the amorphous diamond-like film is deposited. At the pressures and temperatures of diamond-like film deposition (typically, about 0.13 Pascals (Pa) to about 133 Pa (0.001 to 1.0 Torr) (all pressures stated herein are gauge pressure) and less than 50°C), the carbon-containing substances and substances from which an optional additional component may be obtained will be in their vapor form.

For the deposition of carbon and hydrogen in a diamond-like film, hydrocarbons are particularly preferred, including acetylene, methane, butadiene, benzene, methylcyclopentadiene, pentadiene, styrene, naphthalene, and azulene. Mixtures of these hydrocarbons may also be used. Gases containing optional additional components can

also be introduced into the reaction chamber. Gases with low ionization potentials, i.e., 10 eV or less, typically are used for efficient deposition of the diamond-like film.

The additional optional diamond-like film components, including one or more of hydrogen, nitrogen, oxygen, fluorine, silicon, sulfur, titanium, or copper, may be introduced in vapor form into the reaction chamber during the deposition process. Typically, even when the sources for the additional components are solids or fluids, the reduced pressure in the deposition chamber will cause the source to volatilize. Alternatively, the additional components may be entrained in an inert gas stream. The additional components may be added to the chamber while a carbon- or hydrocarbon-containing gas is sustaining the plasma and/or may be added to the chamber after the flow of carbon or hydrocarbon-containing gas has been stopped.

Sources of hydrogen include hydrocarbon gases and molecular hydrogen (H_2). Sources of fluorine include compounds such as carbon tetrafluoride (CF_4), sulfur hexafluoride (SF_6), perfluorobutane (C_4F_{10}), C_2F_6, and C_3F_8. Sources of silicon include silanes such as SiH_4, Si_2H_6, tetramethylsilane, and hexamethyldisiloxane. Sources of oxygen include oxygen gas (O_2), hydrogen peroxide (H_2O_2), water (H_2O), and ozone (O_3). Sources of nitrogen include nitrogen gas (N_2), ammonia (NH_3), and hydrazine (N_2H_6). Sources of sulfur include sulfur hexafluoride (SF_6), sulfur dioxide (SO_2), and hydrogen sulfide (H_2S). Sources of copper include copper acetylacetonate. Sources of titanium include titanium halides such as titanium tetrachloride.

The electrodes may be the same size or different sizes. If the electrodes are different sizes, the smaller electrode will have a larger ion sheath (regardless of whether it is the grounded or powered electrode). This type of configuration is referred to as an "asymmetric" parallel plate reactor. An asymmetric configuration produces a higher voltage potential across the ion sheath surrounding the smaller electrode. Establishing a large ion sheath on one of the electrodes is preferred for this invention because the substrate is preferably located within an ion sheath to benefit from the ion bombardment effects that occur within the sheath.

Preferred electrode surface area ratios are from 2:1 to 4:1, and more preferably from 3:1 to 4:1. The ion sheath on the smaller electrode will increase as the ratio increases, but beyond a ratio of 4:1 little additional benefit is achieved. The reaction chamber itself can act as an electrode. A preferred configuration for this invention

includes a powered electrode within a grounded reaction chamber that has two to three times the surface area of the powered electrode.

In an RF-generated plasma, energy is coupled into the plasma through electrons. The plasma acts as the charge carrier between the electrodes. The plasma can fill the entire reaction chamber and is typically visible as a colored cloud. The ion sheath appears as a darker area around one or both electrodes. In a parallel plate reactor using RF energy, the applied frequency is preferably in the range of about 0.001 Megaherz (MHz) to about 100 MHz, preferably about 13.56 MHz or any whole number multiple thereof. This RF power creates a plasma from the gas (or gases) within the chamber. The RF power source can be an RF generator such as a 13.56 MHz oscillator connected to the powered electrode via a network that acts to match the impedance of the power supply with that of the transmission line and plasma load (which is usually about 50 ohms so as to effectively couple the RF power). Hence this is referred to as a matching network.

The ion sheath around the electrodes causes negative self-biasing of the electrodes relative to the plasma. In an asymmetric configuration, the negative self-bias voltage is negligible on the larger electrode and the negative bias on the smaller electrode is typically in the range of 100 to 2000 volts. While the acceptable frequency range from the RF power source may be high enough to form a large negative direct current (DC) self bias on the smaller electrode, it should not be high enough to create standing waves in the resulting plasma, which is inefficient for the deposition of a diamond-like film.

For planar substrates, deposition of diamond-like films can be achieved in a parallel plate reactor by placing the substrates in direct contact with a powered electrode, which is made smaller than the grounded electrode. This allows the substrate to act as an electrode due to capacitive coupling between the powered electrode and the substrate. This is described in M.M. David et al., AIChE Journal, 37, No. 3, p. 367 (1991). In the case of an elongate substrate, the substrate is optionally pulled through the vacuum chamber continuously while a continuous RF field is placed on the electrode and sufficient carbon-containing gas is present within the chamber. A vacuum is maintained at the inlet and exit of the chamber. The result is a continuous carbon-rich film on an elongated substrate, and substantially only on the substrate.

Methods of Optional Functionalization

The diamond-like film need not be functionalized in order to affix reactants thereto. However, depending on the mode of affixation, it may be desirable to functionalize the silicon-containing layer to create linking agents.

The type of functionalization will depend on the type of reactant(s). Preferably, a variety of conventional approaches to rendering the surfaces of silica (e.g., glass) materials chemically reactive are known and may be employed in the present invention to the extent their use creates linking agents on the substrate for subsequent affixation of reactants. These include using silane coupling agents such as amino silanes to provide amino functionality, carboxy silanes to provide carboxy functionality, epoxy silanes to provide epoxy functionality, mercapto silanes (e.g., those of the formula HS-L-Si(X)(Y)(Z) wherein L is divalent organic linking group, X is a hydrolyzable group such as alkoxy, acyloxy, amine or chlorine, Y and Z are hydrolyzable or nonhydrolyzable groups) to provide mercapto functionality, hydroxy silanes, such as glycidoxypropyl silanes, to provide hydroxy functionality, and the like. Conditions of such silylation reactions (i.e., silanization reactions) are generally known to one of skill in the art. Examples of other silylation reactions are described in Van Der Voort et al., J. Liq. Chrom. & Rel Rechnol., 19, 2723-2752 (1996); Sudhakar Rao et al., Tet. Lett., 28, 4897-4900 (1987); Joos et al., Anal. Biochem., 247, 96-101 (1997); Aebersold et al., Anal. Biochem., 187, 56-65 (1990); and International Publication No. WO 98/39481, published September 11, 1998.

Reactants are introduced preferably for affixation to the linking agents to create binding sites. The modes of affixation may include, without limitation, physical means, such as for example, physically entrapping the reactants within the diamond-like film. In a preferred embodiment of the present invention, reactants are introduced to be affixed to the diamond-like film using linking agents affixed to the diamond-like film.

The devices of the present invention, preferably with affixed reactants, may be used for the separation, detection, and measurement of the species present in samples of biological, ecological, or chemical interest. Of particular interest are macromolecules such as proteins, peptides, saccharides and polysaccharides, genetic materials such as nucleic acids, carbohydrates, cellular materials such as bacteria, viruses, organelles, cell fragments, metabolites, drugs, and the like, and combinations thereof. Of particular interest are the group of macromolecules that are associated with the genetic materials of

living organisms. These include nucleic acids and oligonucleotides such as RNA, DNA, their fragments and combinations, chromosomes, genes, as well as fragments and combinations thereof.

The following examples have been selected merely to further illustrate features, advantages, and other details of the invention. It is to be expressly understood, however, that while the examples serve this purpose, the particular ingredients and amounts used as well as other conditions and details are not to be construed in a matter that would unduly limit the scope of this invention.

Plasma Reactor Descriptions

Reactor One: Diamond-Like Glass (DLG) films were deposited in a home-built plasma reactor designed specifically to deposit on fibers as depicted in Figure 4. The reactor includes a vertical aluminum chamber having two linear aluminum electrodes that are nominally 610 mm (24 inches) long and 38 mm (1.5 inches) wide, located along the linear axis of the chamber, one above the other in a staggered arrangement. The sides and backside of the electrode are insulated and capped off with a ground plane so that only the front side of the electrode is actively exposed to the plasma. The electrodes are powered by a 1.0 kW RF power supply that was operated at a frequency of 13.56 MHz (Model RF 10S form RF Power Products, Kresson, New Jersey) and matching network (Model CPM-1000 from Comdel Inc., Beverly, Massachusetts) and controller (Model MatchPro CPM from Comdel Inc.). The feed gas or mixture of gases was introduced into the deposition chamber through mass flow controllers (from MKS Instruments, Andover, Massachusetts) and was pumped by a roots blower (Model EH1200 from Edwards High Vacuum, Sussex, England) backed by a mechanical pump (Model E2M80 from Edwards High Vacuum). Pressure in the chamber was measured by a capacitance manometer and controlled by a throttle valve and controller (Models 653 and 600 series, respectively, from MKS Instruments).

Reactor Two: A commercial parallel-plate capacitively coupled plasma reactor (commercially available as Model 2480 from PlasmaTherm of St. Petersburg, Florida) was modified and used for the deposition of DLG onto capillary tubes. The reactor is depicted in Figure 5. This reactor includes a grounded chamber electrode containing a powered electrode. The chamber is cylindrical in shape with an internal diameter of 26 inches and height of 12 inches. A circular electrode having a diameter of 55.9 cm (22 inches) was

mounted inside and attached to a matching network and a 3 kW RF power supply that was operated at a frequency of 13.56 MHz. The chamber was pumped by a roots blower backed by a mechanical pump. Unless otherwise stated, the base pressure in the chamber was 0.67 Pa (5 mTorr). Process gases were metered into the chamber either through a mass flow controllers or a needle valve. All the plasma depositions and treatments were done with the substrate located on the powered electrode of the plasma reactor.

Example 1

This example demonstrate the application of a diamond-like film disposed on at least a portion of the substrate which imparts low fluoresence and mechanical strength properties of DLG thin films on glass capillaries. Experimental glass capillaries composed of pure silica glass, drawn from a silica tube, to a capillary with an OD of 200 microns and ID of 50 microns. As part of the draw process, this silica capillary is coated with an acrylated urethane (available from DSM Desotech Inc., Elgin, IL) to a diameter of 300 microns. The acrylate coating was acid stripped by dipping a 19-cm section of capillary sequentially into fuming sulfuric acid (185° C) and water that were poured into two separate beakers. The section of capillary was in each liquid for about 30 seconds. The sectionally stripped glass capillaries were mounted to a sample holder with the stripped section located in free-span and thus not making mechanical contact to any other surface. The sample holder was mounted against the powered electrode of Plasma Reactor One described above. The surface of the capillary facing away from the electrode was pre-cleaned using oxygen plasma at 13.3 Pa (100 mTorr) and 400 Watts for 15 seconds. After cleaning the first side, the chamber was opened, the holder was flipped around, the chamber was closed and the other side of the capillary was similarly pre-cleaned. After oxygen plasma cleaning, DLG films were deposited on the surfaces of the fibers by exposing each side of the fiber to a second plasma for 10 minutes. The second plasma was formed from a mixture of tetramethylsilane (TMS) and oxygen. The flow rate of TMS and oxygen were 150 standard cubic centimeters (sccm) and 100 sccm, respectively. The pressure and RF power were maintained at 40 Pa (300 mTorr) and 200 Watts respectively. The RF power was pulsed at a duty cycle of 90% duty cycle at 10 Hz pulsing frequency. DLG films were deposited for five minutes on each side of the. Mechanical strength of the capillaries was tested using a Vytran proof tester (Model PTR-100, available from Vytran Corporation, Morganville, New Jersey). In order to simulate mechanical handling, the acid

stripped section was wiped once with fingers. The capillaries were mounted in the Vytran Tester and the ultimate strength recorded. In the case where the maximum load was inadequate to break the capillaries, the maximum load was recorded and the actual strength of the capillaries is higher than the recorded value. The mechanical strength results are summarized in Fig. 6. Without any coating, the glass capillaries are prone to fracture whereas, excepting for one sample (strength may have dropped due to a special cause such as contact with the beaker during acid stripping), all the capillary samples failed to break at the testing limit of the proof tester.

The efficacy of the DLG encapsulated glass capillary for capillary electrophoresis is demonstrated by the lack of fluoresence when imaged in a fluoresence microscope. The coating was confirmed to be nominally 2 microns thick based on growth rate measurements made on glass slides with a stylus profilometer: Tencor Instruments, Model No. AS500, Mountainview, CA. A dramatic difference in the intensity of fluoresence may be seen, with the DLG encapsulated fiber displaying little if any fluoresence.

The benefits of the DLG encapsulated capillaries were further quantified by making fluoresence measurements with a Raman spectrometer. The samples were further analyzed using the Renishaw system 1000 (Renishaw Instruments, Model 1000, Gluocestershire, UK). The laser excitation was with an Argon Ion laser operating at 488nm. The 20X objective was used and a single scan was taken on each sample. In addition to the DLG encapsulated capillaries, bare quartz substrate and acrylate encapsulated capillaries were also evaluated for comparison and the results are summarized in Fig. 7. This measurement demonstrates that above 3000 cm^{-1}, the magnitude of fluoresence is less than 200 counts for both DLG enacapsulated and bare quartz whereas it is higher than 30000 counts for the acrylate coated capillary.

The results of this example demonstrate a glass capillary with good mechanical strength durability with little or no fluoresence.

Example 2

This example illustrates the utility of a hydrophilic DLG film in a microfluidic device involving microchanneled polymer plates. Applications of microfluidic devices include the transport of biological fluids, heat transfer fluids, low-friction/drag surfaces, etc. In this example, the substrate was an experimental polymethylmethacrylate (PMMA)

plate having microchannels for transporting liquids including water. The microchanneled polymer plate was prepared by molding poly(methylmethacrylate) sheet (Plexiglass™ DR101 from Rohm and Haas Co of Philadelphia , PA) against a nickel molding tool containing ribs and reservoirs that correspond to the channel and reservoirs in the polymer plate. The tool measured 26.5 cm by 26.5 cm. The sheet of DR101 (nominally 250 μm thick) and molding tool were brought into contact with each other at a temperature of 187°C at a pressure of 6.3×10^5 Pascal for 2 minutes, after which the pressure was increased to 3.2×10^6 Pascal for 2.5 minutes. Thereafter the temperature was decreased to nominally 50°C, and the mold and sheet were then separated.

Using Plasma Reactor Number Two described above, the microchanneled PMMA plate surface was primed initially with an oxygen plasma for 60 seconds at a pressure and RF power of 50 mTorr and 500 Watts, respectively. The flow rates of TMS and oxygen for Sample A were 24 sccm (standard cubic centimeters per minute) and 750 sccm, respectively. One side of the PMMA surface having the channels was treated for five minutes resulting in a DLG thin film thickness of 600 nanometers determined with a Tencor Instruments stylus profilometer. The surface layer of Sample A was further processed to convert the DLG surface to a hydrophilic surface by exposing it to an oxygen plasma at a power and pressure of about 50 mTorr and about 500 Watts, respectively, for 2 minutes. The surface was completely wettable to water, with a contact angle of less than 10 degrees.

Example 3

This example illustrates the moisture barrier properties of DLG films imparted to polymeric capillaries.

A capillary with O.D. of about 360 microns and I.D. of 50 microns was prepared from the polymer Zeonex 480R (Zeon Chemicals L.P.,4100 Bells Lane, Louisville, Kentucky 40211, U.S.A.) in the homebuilt plasma reactor (Reactor No. 1) . The outer surfaces of the capillaries was primed with and oxygen plasma for 2 minutes on each side at a pressure and RF of 100 mTorr and 400 Watts, respectively. The flow rates of TMS and oxygen were 150 sccm and 100 sccm, respectively, resulting in a ratio of TMS to oxygen of 1.5. The pressure and power maintained at 40 Pa (300 mTorr) and 200 Watts respectively. The plasma was operated in a pulsed mode, the pulsing frequency and duty

cycle were maintained at 10 Hz and 90%, respectively. Each side of the capillary was exposed to the plasma for five minutes, resulting in a DLG thin film thickness of about 3 microns. The resulting DLG films were optically clear and did not crack or delaminate when the capillaries were bent and flexed.

The DLG thin film prevented the evaporation of water that was stored in the capillary. A 50 cm piece of the treated and untreated capillary were presoaked with water by pumping water through them with a syringe pump for at least one day. They were then filled with a solution of 10 μg/mL of fluorescein in a 20mM AMPSO, 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid, C.A.S. registry number 68399-79-1, Sigma Chemical Co., St. Louis, MO 63178 buffer at pH 9.0 and then sealed at both ends with an epoxy glue (No. 04001, Elementis Performance Polymers, Bellevue, New Jersey 07109). The evaporation of water could then be observed by monitoring the shrinkage of the volume of liquid inside the capillary using a fluorescent microscope. It was observed that the liquid in the untreated capillary shrunk by evaporation through the capillary wall at a rate almost 30 times that of the treated capillary (with a DLG film thereon).

Without the DLG film, the water evaporates by transport through the walls of the capillary. This result demonstrates the excellent barrier properties of the DLG thin film.

WHAT IS CLAIMED IS

1. A fluid handling device comprising a substrate and an optically transmissive diamond-like film disposed on at least a portion of the substrate.

2. The fluid handling device of claim 1 comprising a capillary having an internal surface and an external surface, wherein at least a portion of at least one of the internal or external surfaces includes an optically transmissive diamond-like film disposed thereon.

3. The fluid handling device of claim 2 wherein the external surface of the capillary includes an optically transmissive diamond-like film disposed on at least a portion thereof.

4. The fluid handling device of claim 1 wherein the diamond-like film comprises at least about 25 atomic percent carbon, from 0 to about 50 atomic percent silicon, and from 0 to about 50 atomic percent oxygen, on a hydrogen-free basis.

5. A fluid handling device comprising a microfluidic article comprising a microfluidic handling architecture comprising a fluid handling surface wherein at least a portion of the fluid handling surface includes a hydrophilic diamond-like film disposed thereon.

6. A fluid handling device comprising a substrate and an optically transmissive and hydrophilic film comprising at least about 25 atomic percent carbon, from 0 to about 50 atomic percent silicon, and from 0 to about 50 atomic percent oxygen, on a hydrogen-free basis, disposed on at least a portion of the substrate.

7. A fluid handling device comprising a substrate and a film comprising at least about 30 atomic percent carbon, at least about 25 atomic percent silicon, and less than about 45 atomic percent oxygen, on a hydrogen-free basis, disposed on at least a portion of the substrate.

8. The fluid handling device of claim 7 comprising a capillary having an internal surface and an external surface, wherein at least a portion of at least one of the internal or external surfaces has the film disposed thereon.

9. The fluid handling device of claim 8 wherein at least a portion of the external surface of the capillary has the film disposed thereon.

10. A method of manufacturing a hydrophilic diamond-like film, the method comprising treating a diamond-like film in an oxygen-containing plasma.

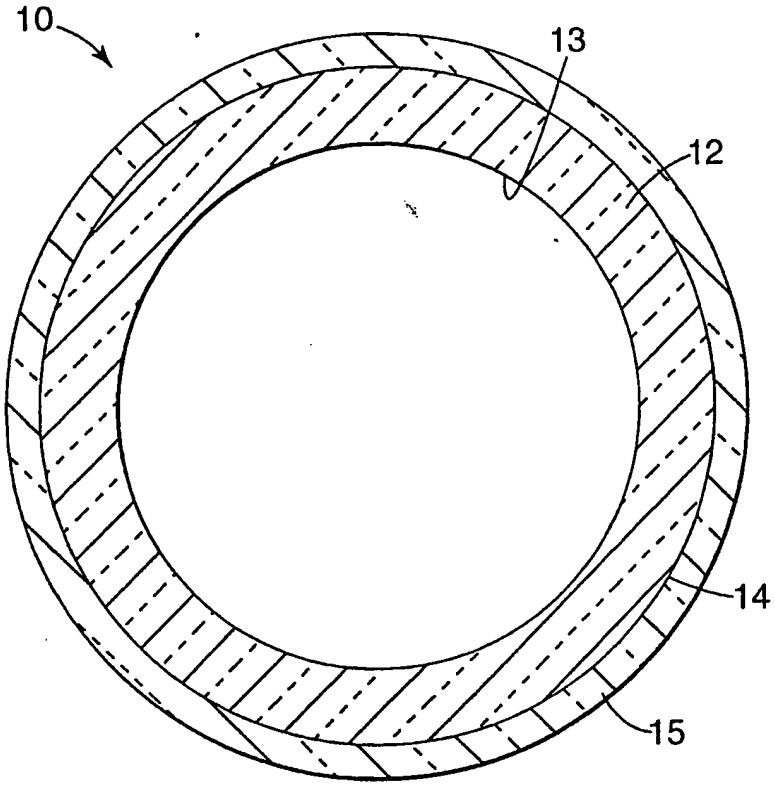


Fig. 1

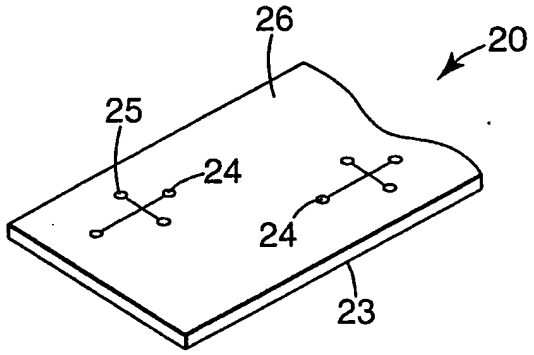


Fig. 2

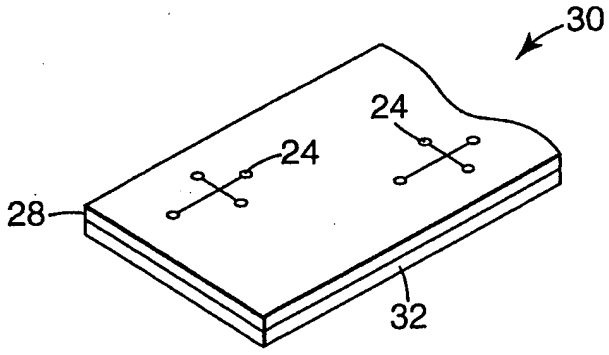


Fig. 3

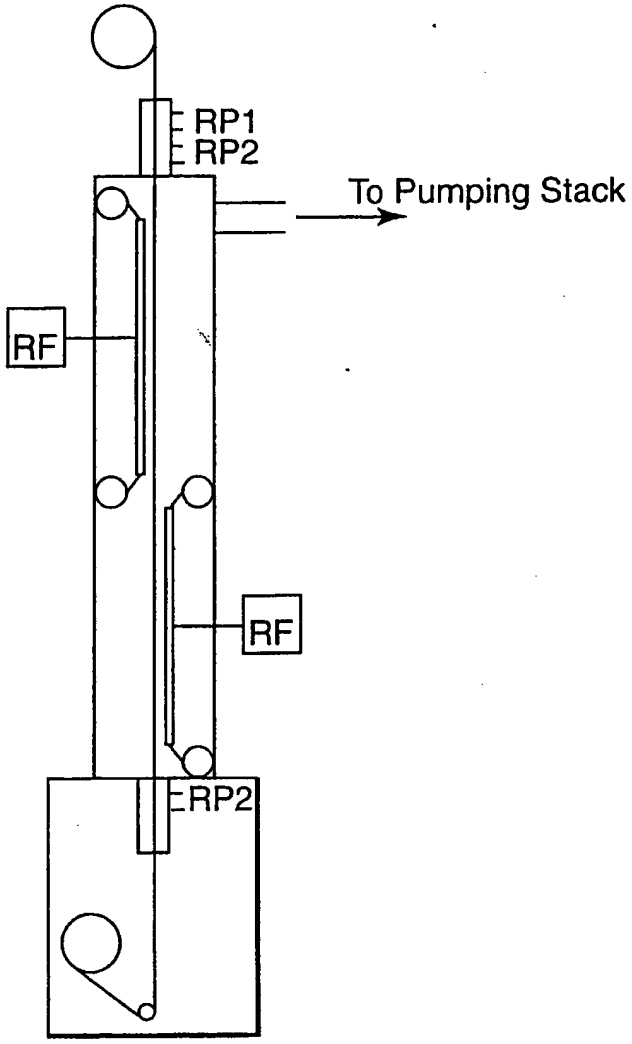


Fig. 4

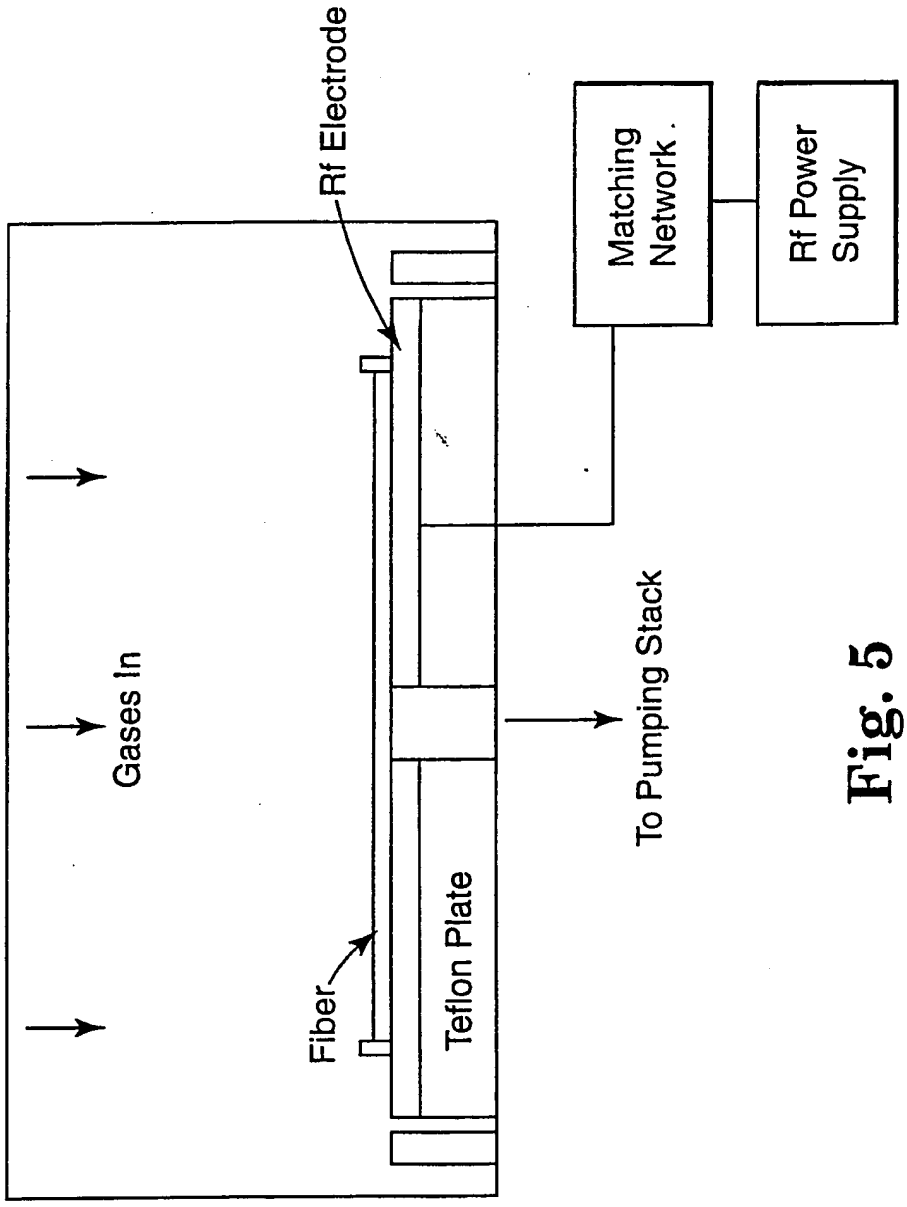

Rf Electrode
Matching Network
Rf Power Supply
Gases In
To Pumping Stack
Fiber
Teflon Plate

Fig. 5

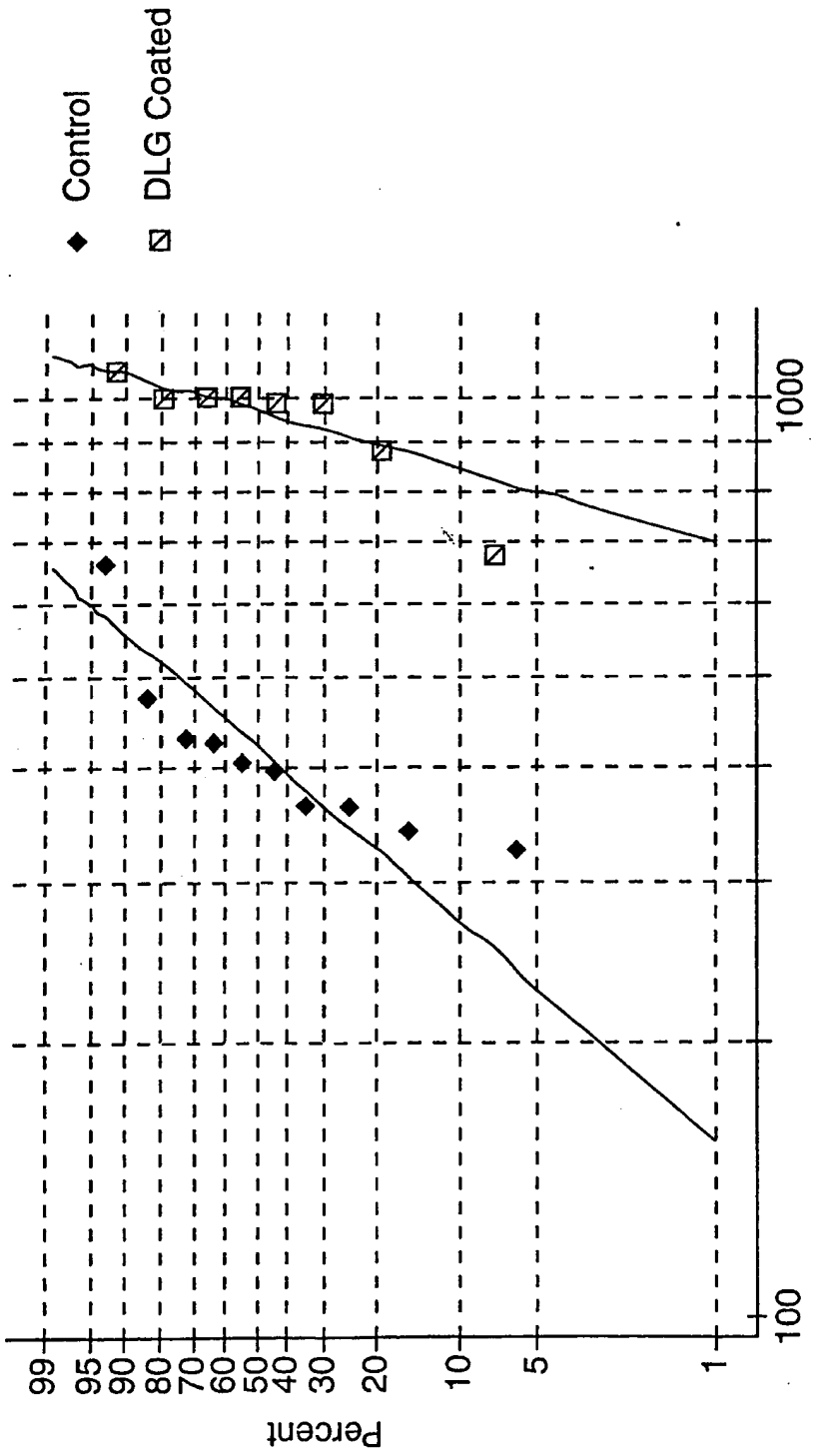
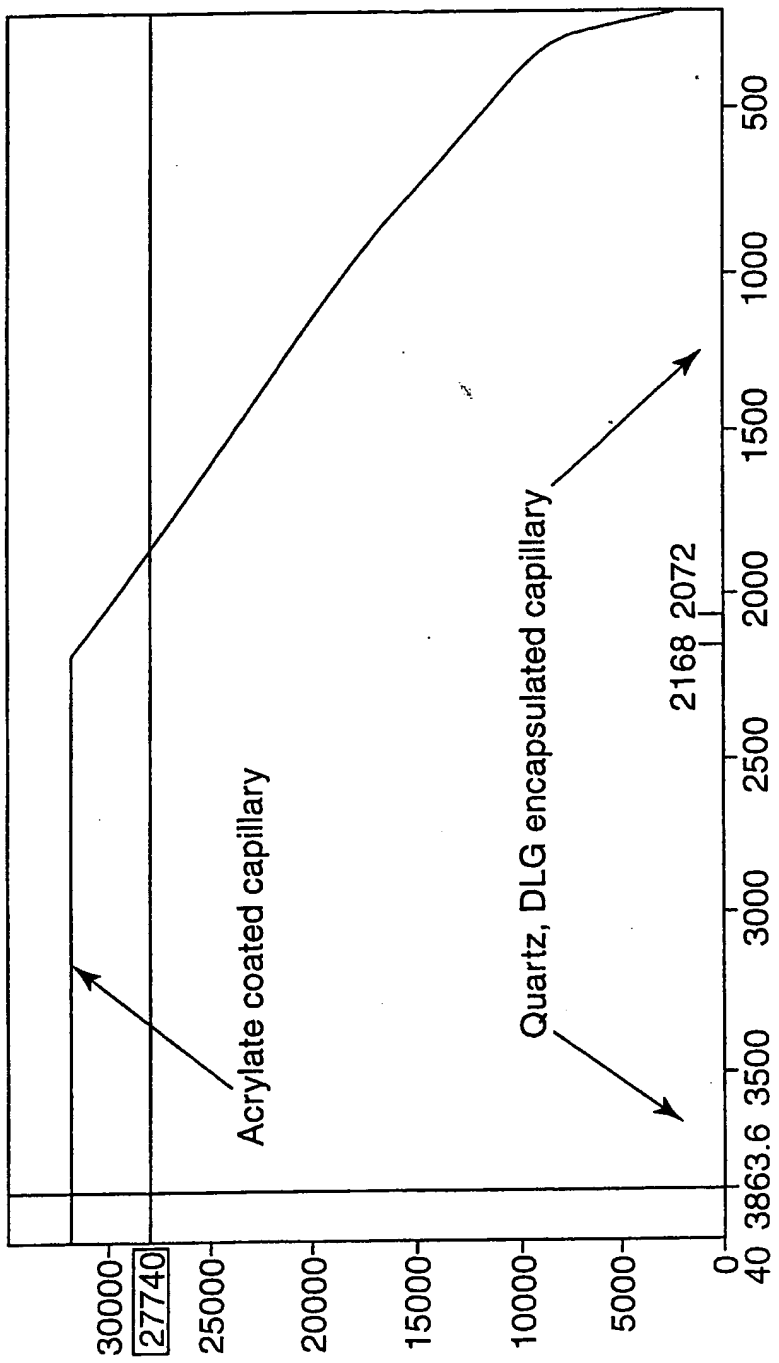

Control
DLG Coated
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
100
1000

Fig. 6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27740
25000
20000
15000
10000
5000
0
40
3863.6
3500
3000
2500
2168
2072
2000
1500
1000
500
Acrylate coated capillary
Quartz, DLG encapsulated capillary

Fig. 7